05-30-00

A

JC836 U.S. PTO
05/26/00

Practitioner's Docket No. 701826/50750 *PATENT*

JC625 U.S. PTO
09/579548
05/26/00

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

NEW APPLICATION TRANSMITTAL

Transmitted herewith for filing is the patent application of
Inventor(s):

WARNING: *37 C.F.R. § 1.41(a)(1) points out:*

"(a) A patent is applied for in the name or names of the actual inventor or inventors.

(1) The inventorship of a nonprovisional application is that inventorship set forth in the oath or declaration as prescribed by § 1.63, except as provided for in § 1.53(d)(4) and § 1.63(d). If an oath or declaration as prescribed by § 1.63 is not filed during the pendency of a nonprovisional application, the inventorship is that inventorship set forth in the application papers filed pursuant to § 1.53(b), unless a petition under this paragraph accompanied by the fee set forth in § 1.17(i) is filed supplying or changing the name or names of the inventor or inventors."

For (title):

CERTIFICATION UNDER 37 C.F.R. 1.10*
*(Express Mail label number is **mandatory**.)*
(Express Mail certification is optional.)

I hereby certify that this correspondence and the documents referred to as attached therein are being deposited with the United States Postal Service on this date May 26, 2000, in an envelope as "Express Mail Post Office to Addressee," mailing Label Number EK571074331US, addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

Patricia W. Turner
(type or print name of person mailing paper)

Patricia W. Turner
Signature of person mailing paper

WARNING: *Certificate of mailing (first class) or facsimile transmission procedures of 37 C.F.R. 1.8 cannot be used to obtain a date of mailing or transmission for this correspondence.*

WARNING:* *Each paper or fee filed by "Express Mail" **must have the number of the "Express Mail" mailing label placed thereon prior to mailing. 37 C.F.R. 1.10(b).*
*"Since the filing of correspondence under § 1.10 without the Express Mail mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will **not** be granted on petition." Notice of Oct. 24, 1996, 60 Fed. Reg. 56,439, at 56,442.*

1. Type of Application

This new application is for a(n)

(check one applicable item below)

[X] Original (nonprovisional)
[ ] Design
[ ] Plant

WARNING: ***Do not*** *use this transmittal for a completion in the U.S. of an International Application under 35 U.S.C. 371(c)(4), unless the International Application is being filed as a divisional, continuation or continuation-in-part application.*

WARNING: ***Do not*** *use this transmittal for the filing of a provisional application.*

NOTE: If one of the following 3 items apply, then complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF A PRIOR U.S. APPLICATION CLAIMED and a NOTIFICATION IN PARENT APPLICATION OF THE FILING OF THIS CONTINUATION APPLICATION.

[ ] Divisional.
[X] Continuation.
[ ] Continuation-in-part (C-I-P).

2. Benefit of Prior U.S. Application(s) (35 U.S.C. 119(e), 120, or 121)

NOTE: A nonprovisional application may claim an invention disclosed in one or more prior filed copending nonprovisional applications or copending international applications designating the United States of America. In order for a nonprovisional application to claim the benefit of a prior filed copending nonprovisional application or copending international application designating the United States of America, each prior application must name as an inventor at least one inventor named in the later filed nonprovisional application and disclose the named inventor's invention claimed in at least one claim of the later filed nonprovisional application in the manner provided by the first paragraph of 35 U.S.C. 112. Each prior application must also be:

(i) An international application entitled to a filing date in accordance with PCT Article 11 and designating the United States of America; or

(ii) Complete as set forth in § 1.51(b); or

(iii) Entitled to a filing date as set forth in § 1.53(b) or § 1.53(d) and include the basic filing fee set forth in § 1.16; or

(iv) Entitled to a filing date as set forth in § 1.53(b) and have paid therein the processing and retention fee set forth in § 1.21(l) within the time period set forth in § 1.53(f).

37 C.F.R. § 1.78(a)(1).

NOTE If the new application being transmitted is a divisional, continuation or a continuation-in-part of a parent case, or where the parent case is an International Application which designated the U.S., or benefit of a prior provisional application is claimed, then check the following item and complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

WARNING: *If an application claims the benefit of the filing date of an earlier filed application under 35 U.S.C. 120, 121*

or 365(c), the 20-year term of that application will be based upon the filing date of the earliest U.S. application that the application makes reference to under 35 U.S.C. 120, 121 or 365(c). (35 U.S.C. 154(a)(2) does not take into account, for the determination of the patent term, any application on which priority is claimed under 35 U.S.C. 119, 365(a) or 365(b).) For a c-i-p application, applicant should review whether any claim in the patent that will issue is supported by an earlier application and, if not, the applicant should consider canceling the reference to the earlier filed application. The term of a patent is not based on a claim-by-claim approach. See Notice of April 14, 1995, 60 Fed. Reg. 20,195, at 20,205.

*WARNING: When the last day of pendency of a provisional application falls on a Saturday, Sunday, or Federal holiday within the District of Columbia, any nonprovisional application claiming benefit of the provisional application **must** be filed prior to the Saturday, Sunday, or Federal holiday within the District of Columbia. See 37 C.F.R. § 1.78(a)(3).*

[X] The new application being transmitted claims the benefit of prior U.S. application(s). Enclosed are ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

3. Papers Enclosed

A. Required for Filing Date under 37 C.F.R. § 1.53(b) (Regular) or 37 C.F.R. § 1.153 (Design) Application

_28_ Pages of Specification
_4_ Pages of Claims
_6_ Sheets of Drawing

*WARNING: **DO NOT** submit original drawings. A high quality copy of the drawings should be supplied when filing a patent application. The drawings that are submitted to the Office must be on strong, white, smooth, and non-shiny paper and meet the standards according to § 1.84. If corrections to the drawings are necessary, they should be made to the original drawing and a high-quality copy of the corrected original drawing then submitted to the Office. Only one copy is required or desired. For comments on proposed then-new 37 C.F.R. 1.84, see Notice of March 9, 1988. (1990 O.G. 57-62).*

NOTE: "Identifying indicia, if provided, should include the application number or the title of the invention, inventor's name, docket number (if any), and the name and telephone number of a person to call if the Office is unable to match the drawings to the proper application. This information should be placed on the back of each sheet of drawing a minimum distance of 1.5 cm. (5/8 inch) down from the top of the page. . ." 37 C.F.R. § 1.84(c)).

(complete the following, if applicable)

[ ] The enclosed drawing(s) are photograph(s), and there is also attached a "PETITION TO ACCEPT PHOTOGRAPH(S) AS DRAWING(S)." 37 C.F.R. § 1.84(b).

[ ] Formal
[X] Informal

B. Other Papers Enclosed

____ Pages of declaration and power of attorney
____ Pages of Abstract
____ Other

4. Additional Papers Enclosed

[X] Amendment to claims

[X] Cancel in this applications claims 2-23 before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)

[X] Add the claims shown on the attached amendment. (Claims added have been numbered consecutively following the highest numbered original claims.)

[X] Preliminary Amendment
[ ] Information Disclosure Statement (37 C.F.R. § 1.98)
[ ] Form PTO-1449 (PTO/SB/08A and 08B)
[ ] Citations
[ ] Declaration of Biological Deposit
[ ] Submission of "Sequence Listing," computer readable copy and/or amendment pertaining thereto for biotechnology invention containing nucleotide and/or amino acid sequence.
[ ] Authorization of Attorney(s) to Accept and Follow Instructions from Representative
[ ] Special Comments
[ ] Other

5. Declaration or Oath (including power of attorney)

NOTE: A newly executed declaration is not required in a continuation or divisional application provided the prior nonprovisional application contained a declaration as required, the application being filed is by all or fewer than all the inventors named in the prior application, there is no new matter in the application being filed, and a copy of the executed declaration filed in the prior application (showing the signature or an indication thereon that it was signed) is submitted. The copy must be accompanied by a statement requesting deletion of the names of person(s) who are not inventors of the application being filed. If the declaration in the prior application was filed under § 1.47 then a copy of that declaration must be filed accompanied by a copy of the decision granting § 1.47 status or, if a nonsigning person under § 1.47 has subsequently joined in a prior application, then a copy of the subsequently executed declaration must be filed. See 37 C.F.R. § 1.63(d)(1)-(3).

NOTE: A declaration filed to complete an application must be executed, identify the specification to which it is directed, identify each inventor by full name, including the family name, and at least one given name without abbreviation together with any other given name or initial, and the residence, post office address and country of citizenship of each inventor, and state whether the inventor is a sole or joint inventor. 37 C.F.R. § 1.63(a)(1)-(4).

[ ] Enclosed

Executed by

(check **all** *applicable boxes)*

[ ] inventor(s).
[ ] legal representative of inventor(s). 37 C.F.R. § 1.42 or 1.43.
[ ] joint inventor or person showing a proprietary interest on behalf of inventor who refused to sign or cannot be reached.

[ ] This is the petition required by 37 C.F.R. § 1.47 and the statement required by 37 C.F.R. § 1.47 is also attached. See item 13 below for fee.

[X] Not Enclosed.

NOTE: Where the filing is a completion in the U.S. of an International Application, or where the completion of the U.S. application contains subject matter in addition to the International Application, the application may be treated as a continuation or continuation-in-part, as the case may be, utilizing ADDED PAGE FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION CLAIMED.

[ ] Application is made by a person authorized under 37 C.F.R. 1.41(c) on behalf of *all* the above named inventor(s).

(The declaration or oath, along with the surcharge required by 37 C.F.R. § 1.16(e), can be filed subsequently).

[ ] Showing that the filing is authorized.
(not required unless called into question. 37 C.F.R. § 1.41(d))

6. Inventorship Statement

WARNING: If the named inventors are each not the inventors of all the claims an explanation, including the ownership of the various claims at the time the last claimed invention was made, should be submitted.

The inventorship for all the claims in this application are:

[ ] The same.

or

[ ] Not the same. An explanation, including the ownership of the various claims at the time the last claimed invention was made,
[ ] is submitted.
[ ] will be submitted.

7. Language

NOTE: An application including a signed oath or declaration may be filed in a language other than English. An English translation of the non-English language application and the processing fee of $130.00 required by 37 C.F.R. § 1.17(k) is required to be filed with the application, or within such time as may be set by the Office. 37 C.F.R. § 1.52(d).

[X] English
[ ] Non-English

[ ] The attached translation includes a statement that the translation is accurate. 37 C.F.R. § 1.52(d).

8. Assignment

[X] An assignment of the invention to Canadian Blood Services

[ ] is attached. A separate [ ] "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or [ ] FORM PTO 1595 is also attached.

[ ] will follow.

NOTE: "If an assignment is submitted with a new application, send two separate letters-one for the application and one for the assignment" Notice of May 4, 1990 (1114 O.G. 77-78).

WARNING: *A newly executed "STATEMENT UNDER 37 C.F.R. § 3.73(b)" must be filed when a continuation-in-part application is filed by an assignee. Notice of April 30, 1993, 1150 O.G. 62-64.*

9. Certified Copy

Certified copy(ies) of application(s)

Canada	2,223,225	28 November 1997
Country	Appln. no.	Filed
Country	Appln. no.	Filed
Country	Appln. no.	Filed

from which priority is claimed

[ ] is (are) attached.

[X] will follow.

NOTE: The foreign application forming the basis for the claim for priority must be referred to in the oath or declaration. 37 C.F.R. § 1.55(a) and 1.63.

NOTE: This item is for any foreign priority for which the application being filed directly relates. If any parent U.S. application or International Application from which this application claims benefit under 35 U.S.C. 120 is itself entitled to priority from a prior foreign application, then complete item 18 on the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

10. Fee Calculation (37 C.F.R. § 1.16)

A. [ ] Regular application

CLAIMS AS FILED

Claims	Number Filed	Basic Fee Allowance	Number Extra	Rate	Basic Fee 37 C.F.R. § 1.16(a) $760.00
~~Total Claims~~ (37 C.F.R. § 1.16(c))		- 20 =	x	$ 18.00	
Independent Claims (37 C.F.R. § 1.16(b))		- 3 =	x	$ 78.00	
Multiple Dependent Claim(s), if any (37 C.F.R. § 1.16(d))			+	$260.00	

[ ] Amendment cancelling extra claims is enclosed.
[ ] Amendment deleting multiple-dependencies is enclosed.
[ ] Fee for extra claims is not being paid at this time.

NOTE: If the fees for extra claims are not paid on filing they must be paid or the claims cancelled by amendment, prior to the expiration of the time period set for response by the Patent and Trademark Office in any notice of fee deficiency. 37 C.F.R. § 1.16(d).

Filing Fee Calculation $_____

B. [ ] Design application
($310.00—37 C.F.R. § 1.16(f))
Filing Fee Calculation $_____

C. [ ] Plant application
($480.00—37 C.F.R. § 1.16(g))
Filing Fee Calculation $_____

11. Small Entity Statement(s)

[ ] Statement(s) that this is a filing by a small entity under 37 C.F.R. §§ 1.9 and 1.27 is (are) attached.

WARNING: *"Status as a small entity must be specifically established in each application or patent in which the status is available and desired. Status as a small entity in one application or patent does not affect any other application or patent, including applications or patents which are directly or indirectly dependent upon the application or patent in which the status has been established. The refiling of an application under § 1.53 as a continuation, division, or continuation-in-part*

(including a continued prosecution application under § 1.53(d)), or the filing of a reissue application requires a new determination as to continued entitlement to small entity status for the continuing or reissue application. A nonprovisional application claiming benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) of a prior application, or a reissue application may rely on a statement filed in the prior application or in the patent if the nonprovisional application or the reissue application includes a reference to the statement in the prior application or in the patent or includes a copy of the statement in the prior application or in the patent and status as a small entity is still proper and desired. The payment of the small entity basic statutory filing fee will be treated as such a reference for purposes of this section." 37 C.F.R. § 1.28(a)(2).

(complete the following, if applicable)

[ ] Status as a small entity was claimed in prior application _____/_____, filed on _____ from which benefit is being claimed for this application under:

35 U.S.C. § [ ] 119(e),
[ ] 120,
[ ] 121,
[ ] 365(c),

and which status as a small entity is still proper and desired.

[ ] A copy of the statement in the prior application is included.

Filing Fee Calculation (50% of **A**, **B** or **C** above) $_____

NOTE: Any excess of the full fee paid will be refunded if a small entity status is established refund request are filed within 2 months of the date of timely payment of a full fee. The two-month period is not extendable under § 1.136. 37 C F.R. § 1.28(a).

12. Request for International-Type Search (37 C.F.R. § 1.104(d))

(complete, if applicable)

[ ] Please prepare an international-type search report for this application at the time when national examination on the merits takes place.

13. Fee Payment Being Made at This Time

[X] Not Enclosed

[ ] No filing fee is to be paid at this time.
(This and the surcharge required by 37 C.F.R. § 1.16(e) can be paid subsequently.)

[ ] Enclosed

[ ] Filing fee $_____

[ ] Recording assignment

($40.00; 37 C.F.R. § 1.21(h))
(See attached "COVER SHEET FOR ASSIGNMENT ACCOMPANYING NEW APPLICATION.") $_____

[ ] Petition fee for filing by other than all the inventors or person on behalf of the inventor where inventor refused to sign or cannot be reached
($130.00; 37 C.F.R. §§ 1.47 and 1.17(i)) $_____

[ ] For processing an application with a specification in a non-English language
($130.00; 37 C.F.R. §§ 1.52(d) and 1.17(k)) $_____

[ ] Processing and retention fee
($130.00; 37 C.F.R. §§ 1.53(d) and 1.21(l)) $_____

[ ] Fee for international-type search report
($40.00; 37 C.F.R. § 1.21(e)) $_____

NOTE: 37 C.F.R. § 1.21(l) establishes a fee for processing and retaining any application that is abandoned for failing to complete the application pursuant to 37 C.F.R. § 1.53(f) and this, as well as the changes to 37 C.F.R. § 1.53 and 1.78(a)(1), indicate that in order to obtain the benefit of a prior U.S. application, either the basic filing fee must be paid, or the processing and retention fee of § 1.21(l) must be paid, within 1 year from notification under § 53(f).

Total Fees Enclosed $_____

14. Method of Payment of Fees

[ ] Check in the amount of $_____.

[ ] Charge Account No. _____ in the amount of $_____.
A duplicate of this transmittal is attached.

NOTE: Fees should be itemized in such a manner that it is clear for which purpose the fees are paid. 37 C.F.R. § 1.22(b).

15. Authorization to Charge Additional Fees

WARNING: *If no fees are to be paid on filing, the following items should <u>not</u> be completed.*

WARNING: *Accurately count claims, especially multiple dependent claims, to avoid unexpected high charges, if extra claim charges are authorized.*

[ ] The Commissioner is hereby authorized to charge the following additional fees by this paper and during the entire pendency of this application to Account No._____.

[ ] 37 C.F.R. § 1.16(a), (f) or (g) (filing fees)

[ ] 37 C.F.R. § 1.16(b), (c) and (d) (presentation of extra claims)

NOTE: Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims cancelled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 C.F.R. § 1.16(d)), it might be best not to authorize the PTO to charge additional claim fees, except possibly when dealing with amendments after final action.

[ ] 37 C.F.R. § 1.16(e) (surcharge for filing the basic filing fee and/or declaration on a date later than the filing date of the application)

[ ] 37 C.F.R. § 1.17(a)(1)-(5) (extension fees pursuant to § 1.136(a).

[ ] 37 C.F.R. § 1.17 (application processing fees)

NOTE: "A written request may be submitted in an application that is an authorization to treat any concurrent or future reply, requiring a petition for an extension of time under this paragraph for its timely submission, as incorporating a petition for extension of time for the appropriate length of time. An authorization to charge all required fees, fees under § 1.17, or all required extension of time fees will be treated as a constructive petition for an extension of time in any concurrent or future reply requiring a petition for an extension of time under this paragraph for its timely submission. Submission of the fee set forth in § 1.17(a) will also be treated as a constructive petition for an extension of time in any concurrent reply requiring a petition for an extension of time under this paragraph for its timely submission." 37 C.F.R. § 1.136(a)(3).

[ ] 37 C.F.R. § 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 C.F.R. § 1.311(b))

NOTE: Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 C.F.R. § 1.311(b)).

NOTE: 37 C.F.R. § 1.28(b) requires "Notification of any change in status resulting in loss of entitlement to small entity status must be filed in the application . . . prior to paying, or at the time of paying, . . issue fee." From the wording of 37 C.F.R. § 1.28(b), (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.

16. Instructions as to Overpayment

NOTE: ". . . Amounts of twenty-five dollars or less will not be returned unless specifically requested within a reasonable time, nor will the payer be notified of such amounts; amounts over twenty-five dollars may be returned by check or, if requested, by credit to a deposit account." 37 C.F.R. § 1.26(a).

[ ] Credit Account No. _____.

[ ] Refund

SIGNATURE OF PRACTITIONER

Reg. No. 34,235

Tel. No.: (617) 345-6057

Customer No.:

David S. Resnick
(type or print name of practitioner)
Nixon Peabody LLP
101 Federal Street
P.O. Address

Boston, MA 02110

[X] **Incorporation by reference of added pages**

(check the following item if the application in this transmittal claims the benefit of prior U.S. application(s) (including an international application entering the U.S. stage as a continuation, divisional or C-I-P application) and complete and attach the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED)

[X] Plus Added Pages for New Application Transmittal Where Benefit of Prior U.S. Application(s) Claimed
Number of pages added _____

[ ] Plus Added Pages for Papers Referred to in Item 4 Above
Number of pages added _____

[ ] Plus added pages deleting names of inventor(s) named on prior application(s) who is/are no longer inventor(s) of the subject matter claimed in this application.
Number of pages added _____

[ ] Plus "Assignment Cover Letter Accompanying New Application"
Number of pages added _____

[ ] **Statement Where No Further Pages Added**

(if no further pages form a part of this Transmittal, then end this Transmittal with this page and check the following item)

[ ] This transmittal ends with this page.

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ADDED PAGES FOR APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED

NOTE: See 37 C.F.R. § 1.78.

17. Relate Back

WARNING: If an application claims the benefit of the filing date of an earlier filed application under 35 U.S.C. 120, 121 or 365(c), the 20-year term of that application will be based upon the filing date of the earliest U.S. application that the application makes reference to under 35 U.S.C. 120, 121 or 365(c). (35 U.S.C. 154(a)(2) does not take into account, for the determination of the patent term, any application on which priority is claimed under 35 U.S.C. 119, 365(a) or 365(b).) For a c-i-p application, applicant should review whether any claim in the patent that will issue is supported by an earlier application and, if not, the applicant should consider canceling the reference to the earlier filed application. The term of a patent is not based on a claim-by-claim approach. See Notice of April 14, 1995, 60 Fed. Reg. 20,195, at 20,205.

(complete the following, if applicable)

[ ] Amend the specification by inserting, before the first line, the following sentence:

A. 35 U.S.C. 119(e)

NOTE: "Any nonprovisional application claiming the benefit of one or more prior filed copending provisional applications must contain or be amended to contain in the first sentence of the specification following the title a reference to each such prior provisional application, identifying it as a provisional application, and including the provisional application number (consisting of series code and serial number)." 37 C.F.R. § 1.78(a)(4).

[ ] "This application claims the benefit of U.S. Provisional Application(s) No(s).:

APPLICATION NO(S).:	FILING DATE
_____/_____	_____"
_____/_____	_____"
_____/_____	_____"

B. 35 U.S.C. 120, 121 and 365(c)

NOTE: "Except for a continued prosecution application filed under § 1.53(d), any nonprovisional application claiming the benefit of one or more prior filed copending nonprovisional applications or international applications designating the United States of America must contain or be amended to contain in the first sentence of the specification following the title a reference to each such prior application, identifying it by application number (consisting of the series code and serial number) or international application number and international filing date and indicating the relationship of the applications. . . . Cross-references to other related applications may be made when appropriate." (See § 1.14(a)). 37 C.F.R. § 1.78(a)(2).

[X] "This application is a

[X] continuation

[ ] continuation-in-part

[ ] divisional

of copending application(s)

[ ] application number 0 /_____ filed on _____"

[X] International Application PCT/CA98/01105 filed on 27 November 1998 and which designated the U.S."

NOTE: The proper reference to a prior filed PCT application that entered the U.S. national phase is the U.S. serial number and the filing date of the PCT application that designated the U.S.

NOTE: (1) Where the application being transmitted adds subject matter to the International Application, then the filing can be as a continuation-in-part or (2) if it is desired to do so for other reasons then the filing can be as a continuation.

NOTE: The deadline for entering the national phase in the U.S. for an international application was clarified in the Notice of April 28, 1987 (1079 O.G. 32 to 46) as follows:

"The Patent and Trademark Office considers the International application to be pending until the 22nd month from the priority date if the United States has been designated and no Demand for International Preliminary Examination has been filed prior to the expiration of the 19th month from the priority date and until the 32nd month from the priority date if a Demand for International Preliminary Examination which elected the United States of America has been filed prior to the expiration of the 19th month from the priority date, provided that a copy of the international application has been communicated to the Patent and Trademark Office within the 20 or 30 month period respectively. If a copy of the international application has not been communicated to the Patent and Trademark Office within the 20 or 30 month period respectively, the international application becomes abandoned as to the United States 20 or 30 months from the priority date respectively. These periods have been placed in the rules as paragraph (h) of § 1.494 and paragraph (i) of § 1.495. A continuing application under 35 U.S.C. 365(c) and 120 may be filed anytime during the pendency of the international application."

[ ] "The nonprovisional application designated above, namely application _____/_____, filed _____, claims the benefit of U.S. Provisional Application(s) No(s).:

APPLICATION NO(S).:	FILING DATE
_____/_____	_____"
_____/_____	_____"
_____/_____	_____"

[ ] Where more than one reference is made above please combine all references into one sentence.

18. Relate Back—35 U.S.C. 119 Priority Claim for Prior Application

The prior U.S. application(s), including any prior International Application designating the U.S., identified above in item 17B, in turn itself claim(s) foreign priority(ies) as follows:

Canada	2,223,225	28 November 1997
Country	Appln. no.	Filed

The certified copy(ies) has (have)

[ ] been filed on _____, in prior application 0 /_____, which was filed on _____.

[ ] is (are) attached.

WARNING: The certified copy of the priority application that may have been communicated to the PTO by the International Bureau may not be relied on without any need to file a certified copy of the priority application in the continuing application. This is so because the certified copy of the priority application communicated by the International Bureau is placed in a folder and is not assigned a U.S. serial number unless the national stage is entered. Such folders are disposed of if the national stage is not entered. Therefore, such certified copies may not be available if needed later in the prosecution of a continuing application. An alternative would be to physically remove the priority documents from the folders and transfer them to the continuing application. The resources required to request transfer, retrieve the folders, make suitable record notations, transfer the certified copies, enter and make a record of such copies in the Continuing Application are substantial. Accordingly, the priority documents in folders of international applications that have not entered the national stage may not be relied on. Notice of April 28, 1987 (1079 O.G. 32 to 46).

19. Maintenance of Copendency of Prior Application

NOTE: The PTO finds it useful if a copy of the petition filed in the prior application extending the term for response is filed with the papers constituting the filing of the continuation application. Notice of November 5, 1985 (1060 O.G. 27).

A. [ ] Extension of time in prior application

*(This item **must** be completed and the papers filed **in the prior application,** if the period set in the prior application has run.)*

[ ] A petition, fee and response extends the term in the pending **prior** application until _____

[ ] A **copy** of the petition filed in prior application is attached.

B. [ ] Conditional Petition for Extension of Time in Prior Application

(complete this item, if previous item not applicable)

[ ] A conditional petition for extension of time is being filed in the pending **prior** application.

[ ] **A copy** of the conditional petition filed in the prior application is attached.

20. Further Inventorship Statement Where Benefit of Prior Application(s) Claimed

(complete applicable item (a), (b) and/or (c) below)

(a) [ ] This application discloses and claims only subject matter disclosed in the prior application whose particulars are set out above and the inventor(s) in this application are

[ ] the same.

[ ] less than those named in the prior application. It is requested that the following inventor(s) identified for the prior application be deleted:

_____

(type name(s) of inventor(s) to be deleted)

(b) [ ] This application discloses and claims additional disclosure by amendment and a new declaration or oath is being filed. With respect to the prior application, the inventor(s) in this application are

[ ] the same.

[ ] the following additional inventor(s) have been added:

_____

(type name(s) of inventor(s) to be deleted)

(c) [ ] The inventorship for all the claims in this application are

[ ] the same.

[ ] not the same. An explanation, including the ownership of the various claims at the time the last claimed invention was made

[ ] is submitted.

[ ] will be submitted.

21. Abandonment of Prior Application *(if applicable)*

[ ] Please abandon the prior application at a time while the prior application is pending, or when the petition for extension of time or to revive in that application is granted, and when this application is granted a filing date, so as to make this application copending with said prior application.

NOTE: According to the Notice of May 13, 1983 (103, TMOG 6-7), the filing of a continuation or continuation-in- part application is a proper response with respect to a petition for extension of time or a petition to revive and should include the express abandonment of the prior application conditioned upon the granting of the petition and the granting of a filing date to the continuing application.

22. Petition for Suspension of Prosecution for the Time Necessary to File an Amendment

WARNING: *"The claims of a new application may be finally rejected in the first Office action in those situations where (1) the new application is a continuing application of, or a substitute for, an earlier application, and (2) all the claims of the new application (a) are drawn to the same invention claimed in the earlier application, and (b) would have been properly finally rejected on the grounds of art of record in the next Office action if they had been entered in the earlier application." MPEP, § 706.07(b), 6th ed., rev.2.*

NOTE: *Where it is possible that the claims on file will give rise to a first action final for this continuation application and for some reason an amendment cannot be filed promptly (e.g., experimental data is being gathered) it may be desirable to file a petition for suspension of prosecution for the time necessary.*

(check the next item, if applicable)

[ ] There is provided herewith a Petition To Suspend Prosecution for the Time Necessary to File An Amendment (New Application Filed Concurrently)

23. Small Entity (37 CFR § 1.28(a))

[ ] Applicant has established small entity status by the filing of a statement in parent application /_____ on _____.

[ ] A copy of the statement previously filed is included.

WARNING: *See 37 CFR § 1.28(a).*

24. NOTIFICATION IN PARENT APPLICATION OF THIS FILING

[ ] A notification of the filing of this
(check one of the following)

[ ] continuation

[ ] continuation-in-part

[ ] divisional

is being filed in the parent application, from which this application claims priority under 35 U.S.C. § 120.

Docket No. 701826/50750

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Canadian Blood Services Group No.:
Serial No.: Continuation of PCT/CA98/01105 Examiner:
Filed: Herewith
For: METHOD FOR INHIBITING IN VIVO IMMUNE RESPONSE

PRELIMINARY AMENDMENT

Before examination, please amend the above-identified Application as follows:

Please cancel claim 1.

Please add the following new claims:

--24. A method for inhibiting an alloimmune response in a patent, comprising the step of administering a therapeutically effective amount of a soluble recombinant human CD40L or a functional fragment thereof containing the active binding site of CD40 and capable of binding thereto.

25. The method of claim 24, wherein the soluble recombinant human CD40L has a sequence comprised in amino acids 108 to 261 of sequence set forth in SEQ ID NO:1.

26. The method of claim 24, wherein the alloimmune response is a human anti-HLA alloimmune response.

27. A method for inhibiting T Cell function in an alloimmune response in a patent, comprising the step of administering a therapeutically effective amount of a soluble recombinant human CD40L or a functional fragment thereof containing the active binding site of CD40 and capable of binding thereto.

28. The method of claim 27, wherein the soluble recombinant human CD40L has a sequence comprised in amino acids 108 to 261 of sequence set forth in SEQ ID NO:1.

29. The method of claim 28, wherein the alloimmune response is a human anti-HLA alloimmune response.

29. The method of claim 28, wherein the alloimmune response is a human anti-HLA alloimmune response.

30. The method of claim 27, for treating or preventing a disease selected from the group consisting of systemic lupus erythematosus (SLE), sjögren's syndrome, sleroderma myositis, Raynaud's syndrome, type 1 diabetes, arthritis and rheumatoid arthritis, inflammatory bowel disease, uveitis, myesthenia gravis, multiple sclerosis, idiopathic thrombocytopenic purpura and graft vs host disease as well as allergies which are dependent on T cells.

31. A method for testing *in vivo* effects of an immunotherapy or inhibition of a human antibody response in an immunodeficient mouse model of human alloimmunization, comprising subjecting the mouse to immunotherapy or inhibition of a human antibody response, said mouse being an immunodeficient mouse and reconstituted with human peripheral blood lymphocytes (PBL) from donors.

32. The method of claim 31, wherein the immunodeficient mouse is γ-irradiated and asialoGM_1 treated for enhancing cellular engraftment.

33. The method of claim 31, wherein the donors are sensitized to HLA antigens.--

REMARKS

Claim 1 has been canceled and new claims 24-33 added. Support for these new claims can be found throughout the specification and their entry is respectfully requested.

Early examination and allowance is respectfully requested.

Respectfully submitted,

5/26/2000

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USE OF A SOLUBLE RECOMBINANT HUMAN CD40L PROTEIN FOR INHIBITING *IN VIVO* IMMUNE RESPONSE

BACKGROUND OF THE INVENTION

(a) Field of the Invention

The invention relates to a method for inhibiting *in vivo* immune response and to the use of a soluble recombinant human CD40L or a sequence within said soluble recombinant human CD40L containing the active binding site with CD40 for inhibiting an immune response. The invention also relates to a mouse model of human alloimmunization for testing *in vivo* effects of an immunotherapy or inhibition of a human antibody response.

(b) Description of Prior Art

Platelet alloimmunization occurs as a result of exposure to "foreign" antigens present in pooled random donor platelet concentrates. A consequence of platelet alloimmunization is the development of a state of refractoriness to subsequent random donor platelet transfusion. Up to 50% of patients with acute leukemia, almost 100% of those with aplastic anemia and 10% of patients with solid tumors, develop platelet alloantibodies. The alloantibodies are most often directed against HLA Class I antigens, although in 10-20% of cases they are directed against platelet-specific antigens such as Pl^A, Bak, Pen. Effective platelet support for such patients is dependent upon provision of compatible platelets selected by HLA matching and/or platelet crossmatching, approaches which are expensive and, in up to one-third of cases, ineffective. A multitude of clinical and experimental studies have indicated that alloimmunization depends upon (or is at least augmented by) the presence of "contaminating" MHC

class II bearing antigen presenting cells (APC) in the transfused blood products.

Investigators have attempted to inactivate donor APC by ultraviolet radiation, or have applied leukofiltration to remove the APC from the transfused product. Most studies, including a large US-based multi-centre study (TRAP, Trial to Reduce Alloimmunization to Platelets) have indicated that the frequency of patients that become alloimmunized is decreased when leukofiltered products are used. However, it is important to note that although these studies reduced the incidence of alloimmunization by approximately 50%, many patients still become alloimmunized.

The major co-stimulatory molecule for B cells is the CD40 molecule. This surface membrane protein which is found on B cells as well as some other cells interacts with a molecule on activated Th cells designated as CD40 ligand (CD40L, gp39 or CD154) CD40-CD40L interaction is critical to B cell activation and differentiation. B cells stimulated with anti-CD40 antibodies undergo transmembrane signaling, cell enlargement, and LFA-1-dependent aggregation. When B cells are stimulated via an appropriate stimuli in combination with anti-CD40, these B cells can proliferate or be induced to isotype switch depending upon the first stimulus. Patients with defective CD40L function have X-linked hyper-IgM syndrome characterized in part by low levels of serum IgG, IgA, and IgE. CD40 and CD40L deficient mice have numerous immune defects including the inability to class switch from IgM to IgG_1 and the inability to stimulate allogenic T cells in an *in vitro* mixed lymphocyte reaction (MLR). Injection of animals with anti-CD40L antibody has been shown to inhibit both a

primary and secondary antibody response, as well as prevent the occurrence of anti-DNA antibodies and disease pathology in lupus-prone mice. Further, administration of a soluble form of CD40L to human B cell hybridomas can induce apoptosis (U.S. Patent No. 5,540,926). While the CD40 molecule on the B cell provides co-stimulation to that cell via interaction with a Th cell expressing the CD40L, it is important to note that the T cell also becomes activated by this mutually synergistic interaction.

It would be highly desirable to be provided with a method to inhibit *in vivo* alloimmunization.

SUMMARY OF THE INVENTION

One aim of the present invention is to provide a method for inhibiting human anti-HLA alloimmune response.

In accordance with the present invention there is provided a soluble recombinant human CD40L or a functional fragment thereof containing the active binding site of CD40 and capable of binding thereto, for inhibiting an immune response. Preferably, the soluble recombinant human CD40L has a sequence comprised in amino acids 108 to 261 of the following sequence:

```
Met Ile Glu Thr Tyr Asn Gln Thr Ser Pro Arg Ser Ala Ala Thr Gly
 1               5                  10                  15

Leu Pro Ile Ser Met Lys Ile Phe Met Tyr Leu Leu Thr Val Phe Leu
            20                  25                  30

Ile Thr Gln Met Ile Gly Ser Ala Leu Phe Ala Val Tyr Leu His Arg
        35                  40                  45

Arg Leu Asp Lys Ile Glu Asp Glu Arg Asn Leu His Glu Asp Phe Val
    50                  55                  60

Phe Met Lys Thr Ile Gln Arg Cys Asn Thr Gly Glu Arg Ser Leu Ser
65                  70                  75                  80
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Leu Leu Asn Cys Glu Glu Ile Lys Ser Gln Phe Glu Gly Phe Val Lys
                85                  90                  95

Asp Ile Met Leu Asn Lys Glu Glu Thr Lys Lys Glu Asn Ser Phe Glu
            100                 105                 110

Met Gln Lys Gly Asp Gln Asn Pro Gln Ile Ala Ala His Val Ile Ser
        115                 120                 125

Glu Ala Ser Ser Lys Thr Thr Ser Val Leu Gln Trp Ala Glu Lys Gly
    130                 135                 140

Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr Leu Glu Asn Gly Lys Gln
145                 150                 155                 160

Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr Ala Gln Val Thr
                165                 170                 175

Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln Ala Pro Phe Ile Ala Ser
            180                 185                 190

Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu Arg Ile Leu Leu Arg Ala
        195                 200                 205

Ala Asn Thr His Ser Ser Ala Lys Pro Cys Gly Gln Gln Ser Ile His
    210                 215                 220

Leu Gly Gly Val Phe Glu Leu Gln Pro Gly Ala Ser Val Phe Val Asn
225                 230                 235                 240

Val Thr Asp Pro Ser Gln Val Ser His Gly Thr Gly Phe Thr Ser Phe
                245                 250                 255

Gly Leu Leu Lys Leu
            260
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SEQ ID NO:1

The immune response inhibited is preferably an alloimmune response, and more preferably a human anti-HLA alloimmune response.

In accordance with the present invention, there is also provided a soluble recombinant human CD40L or a functional fragment thereof containing the active binding site of CD40 and capable of binding thereto, as described above, for inhibiting T cell function. Preferably, the soluble recombinant human CD40L or the functional fragment thereof can be used for treating or preventing T cell dependent or T cell mediated diseases selected from the group consisting of

autoimmune diseases, including systemic lupus erythematosus (SLE), sjögren's syndrome, sleroderma myositis, Raynaud's syndrome, type 1 diabetes, arthritis and rheumatoid arthritis, inflammatory bowel disease, uveitis, myesthenia gravis, multiple sclerosis, idiopathic thrombocytopenic purpura and graft vs host disease as well as allergies which are dependent on T cells.

In accordance with the present invention, there is also provided the use of a soluble recombinant human CD40L or a functional fragment thereof containing the active binding site of CD40 and capable of binding thereto, for the preparation of a medicament for immunotherapy or for treating or preventing a disease selected from the group consisting of SLE, type 1 diabetes, multiple sclerosis, idiopathic thrombocytopenic purpura and graft vs host disease.

Further in accordance with the present invention, there is provided an immunodeficient mouse model of human alloimmunization for testing *in vivo* effects of an immunotherapy or inhibition of a human antibody response, characterized in that the mouse model is a severe combined immunodeficient (SCID) mouse, reconstituted with human peripheral blood lymphocytes (PBL) from donors. Preferably, the donors are sensitized to HLA antigens.

Preferably, the SCID mouse is γ-irradiated and asialoGM_1 treated for enhancing cellular engraftment.

Also in accordance with the present invention, there is provided a method for inhibiting an immune response in a patient, comprising the step of administering a therapeutically effective amount of a soluble recombinant human CD40L or a functional fragment thereof containing the active binding site of CD40 and capable of binding thereto.

In accordance with the present invention, there is also provided a method for inhibiting T cell function in a patient, comprising the step of administering a therapeutically effective amount of a soluble recombinant human CD40L or a functional fragment thereof containing the active binding site of CD40 and capable of binding thereto.

Preferably, the method for inhibiting T cell function is used for treating or preventing T cell dependent or T cell mediated diseases selected from the group consisting autoimmune diseases, including systemic lupus erythematosus (SLE), sjögren's syndrome, sleroderma myositis, Raynaud's syndrome, type 1 diabetes, arthritis and rheumatoid arthritis, inflammatory bowel disease, uveitis, myesthenia gravis, multiple sclerosis, idiopathic thrombocytopenic purpura and graft vs host disease as well as allergies which are dependent on T cells.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1A represents an histogram illustrating the measurements of the total IgG from Hu-PBL-SCID mice on day 18 post-engraftment by ELISA;

Fig. 1B represents a plot chart illustrating the measurements of human alloantibody from Hu-PBL-SCID mice by flow cytometry;

Figs. 2A to 2F illustrate mean ± SEM concentrations of human IgG and IgM in SCID mice reconstituted with PBL from a donor;

Figs. 3A to 3F illustrate flow cytometric analysis of alloantibody production in representative SCID mice reconstituted with PBL from a donor "A" who was previously sensitized to HLA-A2;

Figs. 4A to 4F illustrate flow cytometric analysis of alloantibody production in representative

SCID mice reconstituted with PBL from a donor previously sensitized to HLA-A10;

Fig. 5 illustrates a cumulative comparison of alloantibody responses by flow cytometry;

Fig. 6 illustrates Tetanus toxoid specific antibody production in HLA-A2 challenged 18 KDa-CD154 treated Hu-PBL-SCID mice; and

Fig. 7 illustrates mean + SEM stimulation index of a 4 day 1 way mixed lymphocyte culture.

DETAILED DESCRIPTION OF THE INVENTION

The method of the present invention has been evaluated using a Hu-PBL-SCID mouse model. This immunodeficient mouse model, developed for the present invention, is a valuable model for testing *in vivo* effects of novel immunotherapies or inhibition of the human antibody response.

In accordance with the method of the present invention, it is proposed that anergy induction via inhibition or inappropriate activation of the CD40-CD40L co-stimulatory cascade, is effective in inhibiting an *in vivo* immune response. It is believed that the soluble recombinant CD40L active fragment competes B cell- (or APC-) CD40 interaction with CD40L on the Th cell which disallows the Th cell to be activated to secrete cytokines (such as Th2 cytokines) which thus reduce the transfusion-induced alloimmune response.

To develop an *in vivo* experimental model of human alloimmunization that would be amenable to experimental manipulation, a model was developed, in which mice with severe combined immunodeficiency (SCID) are repopulated with human peripheral blood lymphocytes (Hu-PBL-SCID) from healthy blood donors and challenged with HLA-mismatched lymphocytes.

An *in vivo* model of human alloimmunization was evaluated using severe combined immunodeficient (SCID) mice. SCID mice were irradiated (200 cGy), and reconstituted with human peripheral blood lymphocytes (PBL) from donors sensitized to HLA antigens by prior pregnancy. The reconstituted SCID mice (Hu-PBL-SCID) were then challenged with HLA-mismatched PBL. Alloantibodies were evaluated by flow cytometry (42) and a standard two stage microlymphocytotoxicity (LCT) assay (40).

The Hu-PBL-SCID mice (N=22) that were challenged with PBL expressing the HLA antigens to which the donors had previously been sensitized, made significantly increased levels of both IgM and IgG alloantibodies as compared to unchallenged mice. Responses were measurable by 1 week post reconstitution and challenge. Prior treatment of SCID mice with anti-asialo-GM_1, which depletes murine NK cells and macrophages, further increased the alloantibody response of challenged mice. The human alloantibodies generated were specific for the challenge HLA antigens as assessed by LCT.

Hu-PBL-SCID mice were divided into 4 groups.

Group 1 consists of mice reconstituted with PBL from donor A (as described herein except that 10^7 PBL were used to reconstitute the mice). These animals were bled twice weekly (18 KDa CD40L-untreated and HLA-unchallenged negative control group).

Group 2 consists of mice similar to the ones of Group 1, except that the mice were injected with 200 µg of 18 KDa CD40L via the intraperitoneal route on the day of reconstitution (18 KDa CD40L-treated and HLA-unchallenged negative control group).

Group 3 consists of mice similar to the ones of Group 1, except that the mice also received twice

weekly challenges with HLA-mismatched lymphocytes (18 KDa CD40L-untreated and HLA-challenged positive control group).

Group 4 consists of mice similar to the ones of Group 1, except that the mice were injected with 200 µg of 18 KDa CD40L via the intraperitoneal route on the day of reconstitution and also received twice weekly challenges with HLA-mismatched lymphocytes (18 KDa CD40L-treated and HLA-challenged experimental group).

All mice were examined for total human IgG levels as a measure of the success of functional cellular engraftment as well as to determine if the recombinant soluble 18 KDa CD40L protein product inhibits or affects B cell IgG production. Fig. 1A shows that the total level of human IgG as measured after 18 days of human PBL engraftment is not statistically different in any of the groups. For the purposes of this disclosure, this indicates that the soluble CD40L therapeutic does not adversely affect either cellular engraftment nor does it inhibit the ability of human B cells to produce immunoglobulin. It is therefore unlikely that the CD40L therapeutic induces B cell apoptosis or non-specific B cell anergy when administered *in vivo*. The numbers under each bar indicate the group number corresponding to those of Fig. 1B. Next, in Fig. 1B, the ability of each group of mice to make alloantibody was examined. Groups 1 and 2 did not make significant levels of alloantibody (i.e. no anti-HLA antibody), as expected. Group 3 made enhanced levels of alloantibody, as expected. Group 4 which received a single dose of the soluble 18 Kda CD40L therapeutic made significantly less alloantibody than did group 3.

Administration of a single 200 µg inoculation of soluble recombinant 18 KDa CD40L active component was able to significantly decrease the human alloimmune antibody response to challenge with HLA-mismatched blood cells in a humanized SCID model system. This work therefore shows that *in vivo* administration of a single dose of soluble recombinant CD40L active component can inhibit a specific antibody response. Moreover, T cell proliferation was also prevented *in vitro* in a mixed lymphocyte culture. Therefore, T cell function was also inhibited by the soluble recombinant 18 KDa CD40L active component.

PREPARATION OF A HU-PBL-SCID MOUSE MODEL

SCID Mice

C.B.17 SCID virgin female mice (6-8 weeks of age) were obtained from the Hospital for Sick Children, Toronto, Ontario and were housed under gnotobiotic conditions in the St. Michael's Hospital research vivarium. Blood from the tail vein (300 µl) was collected into untreated microvette tubes (Sarstedt, Montreal, Quebec) and the serum was separated after incubation for 2 h at 22°C. Serum levels of endogenous murine IgG were determined by ELISA and animals with a serum murine immunoglobulin concentration exceeding 10 µg/ml ("leaky" phenotype) were excluded from the study. Commencing 1 week post reconstitution, mice were bled twice weekly for 5 weeks, and weekly thereafter until day 70 post reconstitution.

Reconstituting PBL Donors

Female blood donors with a history of prior pregnancy were screened for evidence of circulating HLA class I alloantibodies. With informed consent, blood samples were obtained at the time of whole blood or platelet apheresis donations and were tested using

a standard NIH microlymphocytotoxicity test against a panel of 30 HLA-typed lymphocytes (40). Donor "A" was blood group O, HLA A1, A3, B7 and B37 positive and had low levels of circulating anti-HLA-A2 and anti-HLA-B5 alloantibodies. Donor "B" was group A, HLA A1, A2, B7 and B8 positive and had low levels of circulating anti-HLA-A10 and anti-B5, -B12 and -B17 alloantibodies.

PBLs from the donor used to reconstitute the Hu-PBL-SCID mice were cultured *in vitro* ($2x10^5$/well) with the same γ-irradiated cells used to challenge the mice ($4x10^5$/well), with or without 18KDa-CD154 for 72 h in a final volume of 200 ml in RPMI-1640 containing 10% fetal calf serum (FCS), 100 U/ml penicillin G, 100 mg/ml streptomycin sulfate, 0.25 mg/ml Amphotericin B as fungizone (Gibco-BRL, Grand Island, NY), 100 mM L-glutamine, and $5x10^{-5}$ M 2-mercaptoethanol (CRPMI), in 96-well flat bottomed tissue culture grade plates at 37°C. Plates were then pulsed with 1 mCi ^{3}H-thymidine for 24 h, wells were harvested onto filter paper and incorporated radioactivity assessed by scintillation counting. For *in vitro* IgG production, plates were cultured with cells as above and were maintained for 18 days by replenishing CRPMI every 3 days. The plates were centrifuged at 300xg for 5 min on day 18 and the supernatant fluid assessed for human IgG levels by ELISA.

Reconstitution

All SCID mice were exposed to 200 cGy of irradiation prior to reconstitution to enhance cellular engraftment. To deplete NK cells, some SCID mice were injected with 20 µl of anti-asialoGM_1 antisera (Wako Pure Chemical Industries LTD, Dallas, TX) 1 day prior to reconstitution. One unit of whole blood was collected into standard collection bags

containing CP2D (Citrate Phosphate Double Dextrose); following centrifugation at 4550 x g for 3.2 min and removal of the supernatant plasma, buffy coats were transferred into a satellite bag. To obtain human PBL, the buffy coat was layered onto a 1.077 g/L Percoll(Pharmacia LKB, Baie d'Urfe, Quebec) gradient and separated by centrifugation (1200 x g for 30 min at 22°C). The PBL were washed three times with phosphate-buffered saline, pH 7.4 (PBS), adjusted to a concentration of $8x10^7$ /ml in 80% FCS in RPMI-1640, and 0.5 ml injected into the peritoneal cavity of recipient SCID mice using a 27 gauge needle.

SCID Mice Challenge

Challenge leukocytes were obtained from heparinized blood and isolated by Percoll density centrifugation as described above. Mice reconstituted with donor "A" cells were challenged with human PBL from HLA-A2 antigen positive blood donors and those reconstituted with donor "B" cells were challenged with human PBL from HLA-A10 antigen positive blood donors. A large number of different donors were used for each challenge; all expressed the pertinent challenge antigen, but expressed a variety of other antigens as well. In a separate experiment, Hu-PBL-SCID mice reconstituted with cells from donor "A" were challenged with cells from 4 individuals expressing only HLA A2, and B5 as antigens foreign to donor "A".

All challenge cells were γ-irradiated with 2500 cGy prior to administration to prevent engraftment in recipient mice. The first challenge consisted of $2x10^7$ PBL/mouse and subsequent immunizations were with 10^7 PBL/mouse. Immunizations with pooled γ-irradiated PBL (in 0.5 ml of 80% fetal calf serum in RPMI-1640) were

performed twice weekly for 3 weeks starting on the day of reconstitution.

Detection of Mouse or Human Immunoglobulin

ELISA plates were coated with 1.25 µg/ml of either goat antimouse or antihuman IgG+IgM (50 µl/well;Caltag-Cedarlane Laboratories, Hornby, Ont.) in 50 mM carbonate/bicarbonate buffer, pH 9.6, for 18 h at 4°C. The plates were then washed three times with washing buffer (0.05% Tween 20/PBS), blocked with 0.2% Tween 20/PBS (200 µl/well) for 2 h at 37°C, and again washed three times with washing buffer. Sera from the mice were serially diluted in PBS, added to the plates (25 µl/well), and incubated for 2 h at 22°C. Serially diluted normal mouse or human serum and purified mouse or human IgG were used as controls and standards. The plates were washed three times in washing buffer and 25 µl of alkaline phosphatase-conjugated $F(ab')_2$ goat antimouse or antihuman IgG or Igm (Cedarlane Laboratories) was added. After incubation at 22°C for 2 h, the plates were washed four times and 100 µl of substrate solution (5 mM p-Nitrophenyl phosphate; BioRad Laboratories, Mississauga, Ontario) was added and absorbance was measured at 405nm. The concentration of IgG and IgM was calculated based upon a standard curve.

Alloantibody Detection

Alloantibodies were detected by flow cytometry as previously described (42) or by a microlymphocytotoxicity test (LCT) (40) using HLA typed target cells. For flow cytometric analysis, SCID sera were diluted 1:10 and incubated with $2x10^5$ fresh HLA typed antigen positive lymphocytes in a volume of 20 µl for 1 h at 22°C. The cells were then washed twice and incubated at 22°C for 1 h in 100 µl each, of 1 µg/ml of affinity-purified fluorescein isothiocyanate

(FITC)-labeled $F(ab')_2$ antihuman IgG, Fc -specific antibody and 0.5 µg/ml of affinity-purified phycoerythrin (PE)-labeled $F(ab')_2$ antihuman IgM, µ specific antibody (Tago; Biosource, Camarillo, CA). The cells were then washed twice and fixed in 1% paraformaldehyde in PBS. Cells were analyzed by flow cytometry as described previously (42). Background staining was assessed by comparison with a serum obtained from each animal prior to any manipulation. Antibody specificity for HLA antigens was confirmed using neat sera in the standard two-stage complement -dependent microlymphocytotoxicity assay using a typed panel of lymphocytes from donors (40); a positive result was defined as > 20% lysis of target cells, unless otherwise stated.

IgG Depletion

Sera from NK-depleted challenged Hu-PBL-SCID mice were pooled and depleted of IgG by affinity chromatography. A saturating quantity of purified goat anti-human IgG, Fc-specific antibody (Atlantic Antibodies, Scarborough ME) was coupled to CNBr-activated sepharose™ 4B media according to the manufacturers directions (Pharmacia Biotech, Baie d'Urfé, PQ). The beads were blocked with excess glycine and extensively washed. Fifty (50) µl of mouse sera was added to 100 µl of packed anti-IgG coated beads with constant mixing for 1 hour at 25°C followed by removal of the supernatant fluid. This IgG-depletion was verified to reduce the IgG content of the pooled sera from 3.2 mg/ml IgG to 20 µg/ml by ELISA. This IgG-depleted sera (referred to as "pooled IgM" in Table 1 below) was then added to another 100 µl of packed fresh anti-IgG coated beads with constant mixing for 1 hour and used in the LCT.

Ten (10) SCID mice were reconstituted by ip injection with $4x10^7$ PBL and were either not further manipulated or received twice weekly ip challenge with HLA-mismatched γ-irradiated lymphocytes for 3 weeks commencing on the day of reconstitution. Challenge lymphocytes were derived from between 8-12 different donors for each challenge. These mice are hereafter referred to as "challenged mice". Both challenged and unchallenged mice made human IgG and IgM immunoglobulin (Figs. 2A and 2B), indicating that the mice were successfully engrafted with human cells. Human IgG levels in unchallenged and in challenged mice reached plateau levels by 14 days post reconstitution and showed little variation until approximately 50 days post reconstitution (Figs. 2A and 2C). Human IgM levels in unchallenged and challenged mice were similar until day 32 post reconstitution (Figs. 2B and 2D).

Total serum human IgG (Figs. 2A, 2C and 2E) and IgM (Figs. 2B, 2D and 2F) were quantitated by ELISA. SCID mice were either reconstituted and not challenged (Figs. 2A and 2B, n=4 mice), reconstituted and challenged with HLA-A2 antigen positive lymphocytes (Figs. 2C and 2D, n=7 mice) or pretreated with anti-asialoGM_1, reconstituted and challenged with HLA-A2 antigen positive lymphocytes (Figs. 2E and 2F, n=14 mice).

SCID mice reconstituted with lymphocytes from donor "A" who had anti-HLA-A2 and -B5 alloantibody were either left unchallenged or challenged with HLA-A2 antigen positive cells. Sera from these mice were tested for allo-reactive IgG and IgM antibody by flow cytometry at each bleed.

Figs. 3A to 3F show reactivity of sera from a typical mouse from each experimental group against

HLA-A2 target lymphocytes. The histograms represent consecutive bleeds over the time period shown by the number of weeks post reconstitution on the right Y-axis. Shorter periods shown indicate that the mice died prior to the next bleed date. Hu-PBL-SCID mice that remained unchallenged made a low level of IgG allo-reactive antibody measurable by day 7 post-reconstitution that did not further increase with time over the study period (Fig. 3A). The unchallenged Hu-PBL-SCID mice did not have allo-reactive IgM antibody detectable by flow cytometry (Fig. 3B). In contrast, as shown by the shift to the right of the histograms, representing increased alloantibody binding, challenged mice made increasing levels of both allo-reactive IgG (Fig. 2, panel C) as well as IgM (Fig. 3D). Both classes of allo-reactive antibody increased until week 3 (Figs. 3C and 3D), after which time the challenge protocol was stopped.

The front histogram in all Figs. 3A to 3F represents reactivity of serum taken from these mice prior to reconstitution (prebleed), and each successive histogram peak is reactivity of serum taken at the indicated time (weeks post reconstitution) as indicated on the right y-axis. Sera (1:10 dilution) were incubated with HLA-A2 antigen positive lymphocytes followed by antihuman IgG (Figs. 3A, 3C and 3E) or antihuman IgM (Figs. 3B, 3D and 3F) fluorochrome-labelled secondary antibody. A shift of the histogram to the right represents increased alloantibody binding. Figs. 3A and 3B show findings in a SCID mouse reconstituted but not challenged, Figs. 3C and 3D in a SCID mouse reconstituted and challenged with HLA-A2 antigen positive lymphocytes, and Figs. 3E and 3F in a SCID mouse pretreated with anti-asialoGM$_1$

to deplete NK cells, reconstituted and challenged with HLA-A2 antigen positive lymphocytes.

Previous reports have suggested that Nk cells are present in SCID mice. To determine if NK-depleted mice could undergo a better alloantibody response, 14 mice were treated with anti-asialoGM$_1$ (depletes NK cells) one day prior to reconstitution and challenge. NK-depleted challenged mice made significantly more total human IgG and IgM, indicating better overall engraftment (Figs. 2E and 2F). These NK-depleted challenged mice also produced higher IgG and IgM class alloantibody reactivity (Figs. 3E and 3F). In addition, these mice attained high steady-state levels of IgG and IgM more rapidly than non-NK-depleted challenged mice (compare Figs. 3E and 3F to 3C and 3D).

Subsequent evaluation of reducing the numbers of reconstituting PBL of donor "A" in an otherwise identical independent experiment, showed that virtually identical results were obtained with 10^7 reconstituting cells (i.e. 4 times less reconstituting cells gave rise to a significant and specific alloantibody response to challenge and the magnitude of alloantibody produced was again significantly increased by prior NK cell depletion).

The specificities of the alloantibodies produced in the anti-asialoGM$_1$ -treated challenged mice were determined by LCT and the results are shown in Table 1 (columns labelled mouse 1, mouse 2, and mouse 3). The LCT demonstrated complement-fixing alloantibodies to HLA-A2, as well as to the A9, and A28 antigens which share and define the 2C public epitope. Alloantibodies to HLA-B5 were also detected as well as to the B17 and B21 antigens which are known to crossreact strongly with B5. The only other

consistent alloantibodies detected were against A10 and B8 which were not detectable in the donors sera nor crossreact with A2 or B5.

To determine the specificity of the IgM alloantibody, the sera from all 3 NK-depleted-challenged mice were pooled and depleted of IgG by two rounds of IgG-specific affinity chromatography. Table 1 shows that the "pooled IgM" class alloantibody reactive with all pertinent HLA-A antigens were observed but B5 was the only HLA-B antigen that reacted with the IgG-depleted sera.

Table 1

Summary of anti-HLA specificity by lymphocytotoxicity testing against a 30 cell panel of sera from NK-depleted challenged mice reconstituted with PBL from donor A*

	pan-HLA challenged				HLA-A2 and B5 challenged			
HLA antigen	Mouse 1	Mouse 2	Mouse 3	Pooled IgM	Mouse 4	Mouse 5	Mouse 6	Mouse 7
A1	-[T]	-	-	-	-	-	-	-
A2	+	+	+	+	+	+	+	+
A3	-	-	-	-	-	-	-	-
A9	+	+	+	+	+	+	-	+
A10	+	+	+	w	-	-	-	-
A11	?	+	?		-	-	-	-
A28	+	+	+	+	+	-	+	+
A30	-	-	-		-	-	-	-
A31	-	-	-	-	-	-	-	-
A34	-	-	-		-	-	-	-
B5	+	+	+	+	+	+	+	+
B7	-	-	-	-	-	-	-	-
B8	?	+	+	-	-	-	-	-
B13	-	-	-		-	-	-	-
B14	-	-	-		-	-	-	-
B16	-	-	-	-	-	-	-	-
B17	+	+	+	-				
B21	+	+	+	-				
B22	-	?	-					
B27	-	-	-		-	-	-	-
B37	-	-	-		-	-	-	-
B40	-	-	-					
B42	-	-	-					
B44	-	-	-		-	-	-	
B75	-	-	-	-	-	-	-	-

* The HLA type of donor "A" was HLA-Al, A3, B7, B37; serum from this donor was also assessed by LCT at the time of engraftment and alloantibody reactive with the A2 and B5 antigens only could be detected.

[T] A negative (-) sign denotes no reactivity with that HLA antigen; a positive (+) sign denotes that sera from that mouse reacted with panel cells expressing the corresponding HLA antigen; ? indicates that reactivity with that HLA antigen could not be verified, w designates a weak response.

Hu-PBL-SCID mice were repopulated with donor "A" lymphocytes in a separate experiment and were challenged with cells expressing HLA-A2 and B5 as the

only HLA antigens foreign to donor "A". These challenged mice made alloantibody against HLA typed panel cells expressing A2, A9, A28, and B5 but not against those expressing A10 or B8 antigens (Table 1, see columns labelled mouse 4 through mouse 7).

PBL from a blood donor with low levels of serum alloantibody to HLA-A10, B5, B12 and B17 (donor "B") were also used to reconstitute mice. Mice were divided into unchallenged, HLA-A10 challenged, and NK-depleted and HLA-A10 challenged using essentially the same protocol as described for donor "A". Sera (1:10 dilution) from unchallenged, challenged, and NK-depleted challenged Hu-PBL-SCID mice were tested for allo-reactive antibody by flow cytometry against HLA-A10 antigen positive target lymphocytes. Unchallenged donor "B" mice made marginal levels of human IgG and no IgM class alloantibody as assessed by reactivity with HLA-A10 target cells (Figs. 4A and 4B). However, antigenic challenge of these mice did provoke an allo-response involving IgG but not IgM class antibody (Figs. 4C and 4D). NK-depleted challenged mice again made a stronger alloantibody response (Figs. 4E and 4F) than non-NK-depleted challenged mice and this "optimization" of the SCID milieu permitted measurable IgM class alloantibody. As with mice reconstituted with PBL from donor "A", the time to alloantibody detection in donor "B" was more rapid in the NK-depleted challenged mice (compare Figs. 4C and 4D to Figs. 4E and 4F). The sera from these mice were subjected to analysis by LCT testing. Alloantibodies were not detected in unchallenged mice but alloantibodies reactive with HLA-A10 were observed in all challenged mice and all anti-asialoGM$_1$-treated-challenged mice.

In Figs. 4A to 4F, sera (1:10 dilution) in this case were incubated with HLA-A10 antigen positive lymphocytes followed by antihuman IgG (Figs. 4A, 4C and 4E) or antihuman IgM (Figs. 4B, 4D and 4F). Figs. 4A and 4B show findings in a SCID mouse reconstituted but not challenged, Figs. 4C and 4D in a SCID mouse reconstituted and challenged with HLA-A10 antigen positive lymphocytes, and Figs. 4E and 4F in a SCID mouse pretreated with anti-asialoGM_1 to deplete NK cells, reconstituted and challenged with HLA-A10 antigen positive lymphocytes. The mice represented in Figs. 4A/4B and 4E/4F died after 2½ and 3½ weeks postreconstitution, respectively.

The cumulative flow cytometric data for all donor "A" and "B" reconstituted SCID mice over 5 experiments against antigen positive cells is shown in Fig. 5. The data from the challenged group is the mean reactivity of sera from 22 mice and the unchallenged group is the mean of 13 mice. All NK-depleted challenged mice (22/22) made alloantibody that reacted with antigen positive challenge cells.

Hu-PBL-SCID mice were unchallenged (□, n=13) or challenged with leukocytes (■, n=22) and assessed for IgG class alloantibody reactivity using typed antigen positive cells. The data on the Y-axis is reported as the mean log fluorescence intensity (± SEM) for all mice.

An *in vivo* model of the secondary immune response was established in a Hu-PBL-SCID mouse model system using lymphocytes from previously sensitized individuals. Intraperitoneal inoculation of SCID mice with these previously sensitized human PBL resulted in engraftment of the mice and challenge with HLA-mismatched lymphocytes resulted in specific allo-antibody formation in all mice. This model was used on

12 donors who were not sensitized to HLA antigens. It was found that HLA-specific antibody was never observed. Therefore this model can also be used for defining persons functionally immunized to an antigen as will be described later.

It is known that SCID mice do not contain functional B cells or T cells. The mice do however possess essentially normal NK cells which can inhibit human lymphocyte engraftment and antibody production, likely by destroying the engrafting cells. It has been shown in the present application that pretreatment with anti-asialoGM_1 to deplete NK cells resulted in greater IgG and IgM production and in specific alloantibody production over that seen in non-NK-depleted challenged mice. Thus, pretreatment of SCID mice with anti-asialoGM_1 did allow maximal alloantibody production.

The lymphocytes used for antigenic challenge of mice reconstituted with PBL from donor "A" were from individuals that were all HLA-A2 antigen positive. Each challenge consisted of lymphocytes derived from between 8-12 different individuals and a different series of individuals was used for each challenge; this was necessary to obtain sufficient PBL for immunization of all mice with the same PBL. Thus, these mice were exposed to lymphocytes from a large number of different donors possessing a wide spectrum of other HLA Class I and Class II antigens in addition to HLA-A2. The response of the mice consisted of alloantibodies to several HLA antigens in addition to A2. The phenomenon of "responders" is well recognized i.e. individuals who make antibody to one challenge are likely to make antibodies to challenge with new immunogenic antigens. These other antibodies could represent primary responses to HLA antigens or

secondary responses of previously undetected antibodies, or alloantibodies that are cross reactive with either A2 or B5 which preexisted in donor "A". The anti-HLA-A alloantibodies produced included anti-A2, -A9, -A10, -A11 and -A28. Since the A9 and A28 antigens are well known to cross react with A2, reactivity with these HLA-A alloantigens is not unexpected. It is however more difficult to account for anti-A10 and A11 reactivity. The anti-A10 and -A11 represent either primary responses, or a secondary response to a putative paternal HLA antigen where the antibody was not initially detectable in the serum of donor "A". Since the paternal HLA typing is unknown, it was not possible to differentiate between these possibilities, however, IgM alloantibody was not produced to the A10 and A11 antigens and therefore the alloantibodies reacting against A10 and A11 are likely an amnestic response. In the case of responses to HLA-B antigens; since donor "A" had pre-existing anti-B5, alloantibody reactive with this antigen is expected. IgM alloantibody reactive with HLA-B antigens could only be detected to B5. Donor A's HLA type was HLA A1, A3, B7 and B37 and none of the mice generated antibodies that reacted with these "self antigens" despite the strong likelihood that the pooled challenge PBL would express these antigens (A1 found in 26% of the Caucasian population, A3 in 25%, and B7 in 22%). This indicates that the mice maintained specificity for foreign antigens without generating auto-reactive antibody. In addition, the fact that challenge with A2- and B5-only expressing cells did not induce formation of A10 and B8 indicates that a generalized immune stimulation was not simply induced but that the allospecificity was maintained for the stimulating cells.

Mice reconstituted with PBL from either donor "A" or "B" made IgM as well as IgG alloantibodies. This is of interest because although IgG is well known to be produced in a secondary response from activation of memory B cell clones, IgM production is not generally considered to be produced by memory B cell clones. Antibodies of the secondary response have however been observed without class switching and experiments with adoptive primary and secondary responses have shown that memory B cells producing IgM can be observed. An alternative explanation may be that these previously sensitized donors possess memory T helper cells to HLA antigens that are able to efficiently activate "naive B cells" to secrete IgM. Coupling of secondary T cell carrier epitopes to primary haptens has been reported to generate primary immunization in Hu-PBL-SCID mice. The T cells most often found in Hu-PBL-SCID mice show the $CD45^{RO}$ memory phenotype and it has been reported that transfer of $CD45^{RO}$, but not $CD45^{RA}$ (naive phenotype), T helper cells could induce purified human B cells in SCID mice to produce immunoglobulin. It is therefore possible that primary IgM-secreting B cells were activated via the memory T cell pool present in the two donors employed for reconstitution of the SCID mice.

To determine if secondary IgG production from memory B cells was a non-specific inhibition by 18 KDa-CD154, the IgG anti-tetanus antibody levels from treated and untreated Hu-PBL-SCID mice were examined. Fig. 6 shows that while stimulation of Hu-PBL-SCID mice with HLA-mismatched lymphocytes caused a slight increase in production of anti-tetanus starting at day 10, the administration of 18 KDa-CD154 did not significantly interfere with the anti-tetanus IgG levels in these four groups of mice ($p>.05$). In all

cases, the reactivity of anti-tetanus IgG in the mice remained well below the levels of anti-tetanus from the donor's serum taken at the time of reconstitution (Fig. 6, dotted line). Hu-PBL-SCID mice as in Fig. 1B were assessed for anti-tetanus toxoid specific antibody. The data on the Y-axis is reported as the mean absorbance (± SEM, n=4 mice per group).

To determine if administration of 18KDa-CD154 was also able to directly affect cell proliferation, responder cells (from the individual providing the PBL for reconstitution) and stimulator cells (challenge cells) in the presence or absence of 18KDa-CD154 were set up as mixed lymphocyte cultures. Addition of γ-irradiated stimulator cells to these cultures induced a 4-fold increase in cell proliferation (Fig. 7). Addition of 18KDa-CD154 to the stimulator + responder mixed lymphocyte cultures prevented the increase in cell proliferation as compared to the absence of 18KDa-CD154 (Fig. 7). The stimulation index was calculated by dividing the cpm of cultured responder cells (R) alone by the cpm of responder cells in the presence of A; unstimulated lymphocytes (R), B; lymphocytes reacted with 18 KDa-CD154 (R + CD154), C: lymphocytes stimulated with γ-irradiated stimulator lymphocytes (R + S), D; lymphocytes stimulated with γ-irradiated stimulator lymphocytes and 18 KDa-CD154 (R + S + CD154).

Accordingly, 18 KDa-CD154 could inhibit alloantibody production by inhibiting T cell activation or T cell function. To test this allegation, mixed lymphocyte cultures were performed and T cell proliferation was shown to be reduced in the presence of 18 KDa-CD154. Since B cells do not play a major role in the mixed lymphocyte culture, T cell activation and proliferation could be directly

inhibited by 18 KDa-CD154 treatment. The alloimmune response to blood transfusion has been suggested to be a Th_2-dominant immune response. Since CD154 is linked to T cell activation along the Th_2 cytokine pathway and T cell proliferation can be defective when IL-4 is absent, the 18KDa-CD154 fragment have decreased T cell proliferation due to decreased Th_2 cytokine production.

It is thus demonstrated in the present application that lymphocytes from individuals previously sensitized to HLA antigens can reconstitute SCID mice and can generate reproducible IgG and IgM allo-immune responses following repeated challenge with selected "foreign" HLA antigens. The development of this model will allow detailed study of the mechanisms of alloimmunization and should facilitate the *in vivo* assessment of new strategies for the modulation of human alloimmunization to blood cell antigens.

It is also demonstrated in the present application that administration of a recombinant 18 KDa-CD154 molecule can inhibit an alloimmune response. This 18 KDa-CD154 molecule may have good therapeutic potential to inhibit human transfusion-induced alloimmunization.

The present invention will be more readily understood by referring to the following examples, which are given to illustrate the invention rather than to limit its scope.

EXAMPLE I

Immunotherapy drug screening design for alloantibody

SCID mice (or other immunodeficient mice such as NOD-SCID, NUDE, etc...) as prepared in accordance with the present invention are engrafted with human PBL from pregnancy sensitized blood donors and

challenged with HLA-mismatched lymphocytes in the presence or absence of any drug or therapeutic (i.e. such as an immunotherapeutic to include IVIG, anti-idiotype antibodies, CTLA4Ig, anti-CD40 ligand antibody, anti-CD40 antibody, anti-CD-4, anti-IL-2 receptor, anti-CD-28, anti-CD-80, anti-CD-86, anti-CD-11A, cyclosporine A, FK506, biologically active peptides such as altered peptide ligands, etc.). The HLA-specific human IgG and IgM were measured as described above.

EXAMPLE II

Evaluation of functional immunization of an individual with an antigen

Immunodificient mice such as SCID mice or NOD-SCID mice as prepared in accordance with the present invention are engrafted with PBL from an individual who has been vaccinated (to polio, tetanus, hepatitis B surface antigen, HIV, cancer cells or cancer antigens, etc.) or not. The Hu-PBL-SCID mice are challenged with the same antigen. The antigen-specific human IgG and IgM produced in the mice are measured. The persons exposed to a functional or protective vaccine make antigen-specific or neutralizing IgG and IgM.

EXAMPLE III

Immunotherapy drug screening design for alloantibody

Immunodificient mice such as SCID mice or NOD-SCID mice as prepared in accordance with the present invention are engrafted with PBL from an individual who has been vaccinated as described in Example II above. The Hu-PBL-SCID mice are challenged with the same antigen in the presence or absence of any drug or therapeutic, such as described in Example I above. The

antigen-specific human IgG and IgM are measured as described above.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. Use of a soluble recombinant human CD40L or a functional fragment thereof containing the active binding site of CD40 and capable of binding thereto, for inhibiting an immune response.

2. The use of claim 1, wherein the soluble recombinant human CD40L has a sequence comprised in amino acids 108 to 261 of sequence set forth in SEQ ID NO:1.

3. The use of claim 2, wherein the immune response is an alloimmune response.

4. The use of claim 3, wherein the alloimmune response is a human anti-HLA alloimmune response.

5. Use of a soluble recombinant human CD40L or a functional fragment thereof containing the active binding site of CD40 and capable of binding thereto, for inhibiting T cell function.

6. The use of claim 5, wherein the soluble recombinant human CD40L has a sequence comprised in amino acids 108 to 261 of sequence set forth in SEQ ID NO:1.

7. The use of claim 6, wherein the immune response is an alloimmune response.

8. The use of claim 7, wherein the alloimmune response is a human anti-HLA alloimmune response.

9. The use of claim 5, for treating or preventing a disease selected from the group consisting of systemic lupus erythematosus (SLE), sjögren's syndrome, sleroderma myositis, Raynaud's syndrome, type 1 diabetes, arthritis and rheumatoid arthritis, inflammatory bowel disease, uveitis, myesthenia gravis, multiple sclerosis, idiopathic thrombocytopenic purpura and graft vs host disease as well as allergies which are dependent on T cells.

10. Use of a soluble recombinant human CD40L or a functional fragment thereof containing the active binding site of CD40 and capable of binding thereto, for the preparation of a medicament for immunotherapy.

11. Use of a soluble recombinant human CD40L or a functional fragment thereof containing the active binding site of CD40 and capable of binding thereto, for the preparation of a medicament for treating or preventing a disease selected from the group consisting of systemic lupus erythematosus (SLE), sjögren's syndrome, sleroderma myositis, Raynaud's syndrome, type 1 diabetes, arthritis and rheumatoid arthritis, inflammatory bowel disease, uveitis, myesthenia gravis, multiple sclerosis, idiopathic thrombo-cytopenic purpura and graft vs host disease as well as allergies which are dependent on T cells.

12. Use of an immunodeficient mouse model of human alloimmunization for testing *in vivo* effects of an immunotherapy or inhibition of a human antibody response, said mouse model being an immunodeficient mouse, reconstituted with human peripheral blood lymphocytes (PBL) from donors.

13. The use of claim 12, wherein the immunodeficient mouse is γ-irradiated and asialoGM$_1$ treated for enhancing cellular engraftment.

14. The use of claim 12, wherein the donors are sensitized to HLA antigens.

15. A method for inhibiting an immune response in a patient, comprising the step of administering a therapeutically effective amount of a soluble recombinant human CD40L or a functional fragment thereof containing the active binding site of CD40 and capable of binding thereto.

16. The method of claim 15, wherein the soluble recombinant human CD40L has a sequence comprised in amino acids 108 to 261 of sequence set forth in SEQ ID NO:1.

17. The method of claim 16, wherein the immune response is an alloimmune response.

18. The method of claim 17, wherein the alloimmune response is a human anti-HLA alloimmune response.

19. A method for inhibiting T cell function in a patient, comprising the step of administering a therapeutically effective amount of a soluble recombinant human CD40L or a functional fragment thereof containing the active binding site of CD40 and capable of binding thereto.

20. The method of claim 19, wherein the soluble recombinant human CD40L has a sequence comprised in

amino acids 108 to 261 of sequence set forth in SEQ ID NO:1.

21. The method of claim 20, wherein the immune response is an alloimmune response.

22. The method of claim 21, wherein the alloimmune response is a human anti-HLA alloimmune response.

23. The method of claim 19, for treating or preventing a disease selected from the group consisting of systemic lupus erythematosus (SLE), sjögren's syndrome, sleroderma myositis, Raynaud's syndrome, type 1 diabetes, arthritis and rheumatoid arthritis, inflammatory bowel disease, uveitis, myesthenia gravis, multiple sclerosis, idiopathic thrombocytopenic purpura and graft vs host disease as well as allergies which are dependent on T cells.

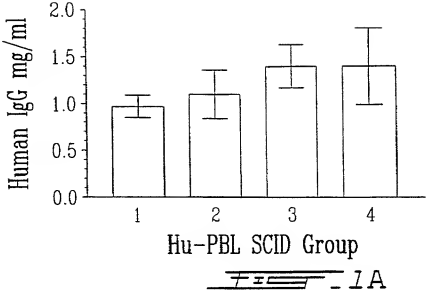


Fig. 1A

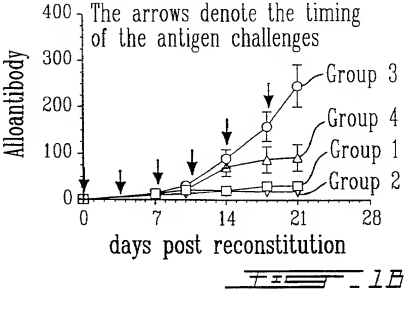


Fig. 1B

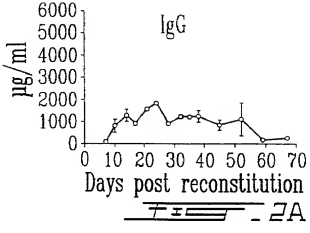


FIG. 2A

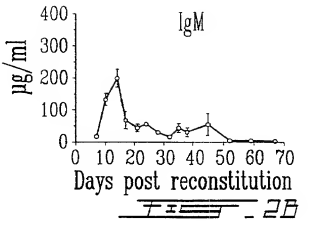


FIG. 2B

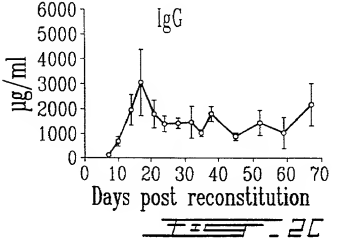


FIG. 2C

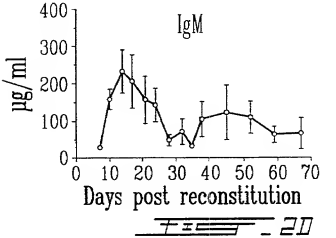


FIG. 2D

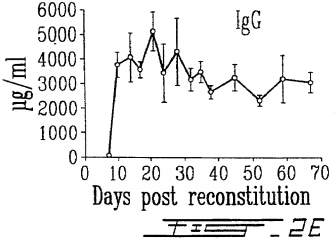


FIG. 2E

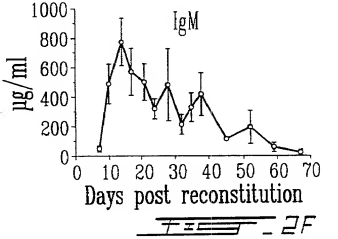


FIG. 2F

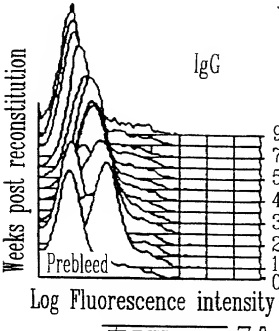


Fig. 3A

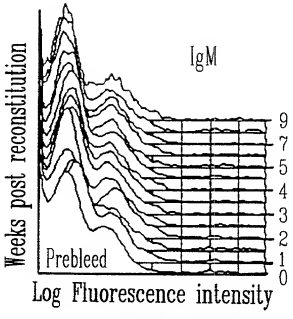


Fig. 3B

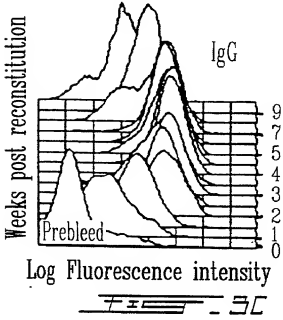


Fig. 3C

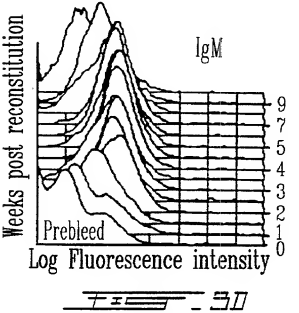


Fig. 3D

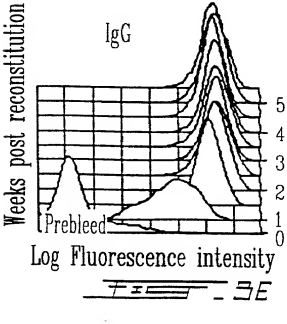


Fig. 3E

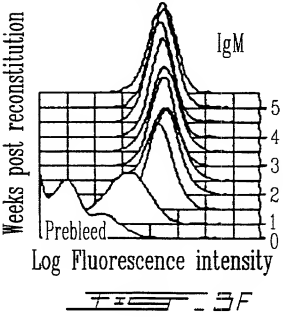


Fig. 3F

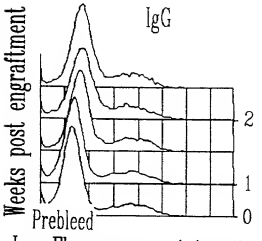


Fig. 4A

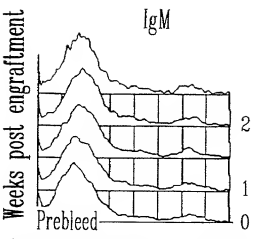


Fig. 4B

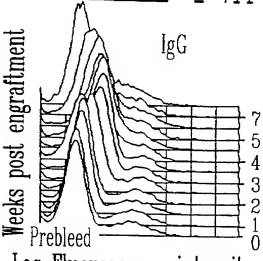


Fig. 4C

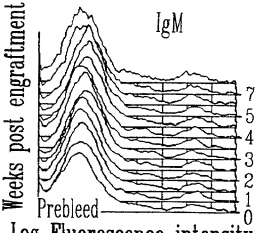
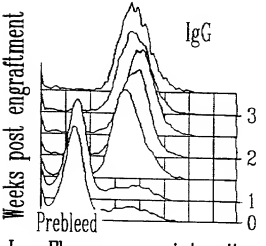


Fig. 4D



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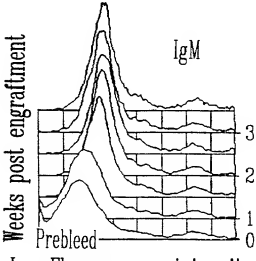

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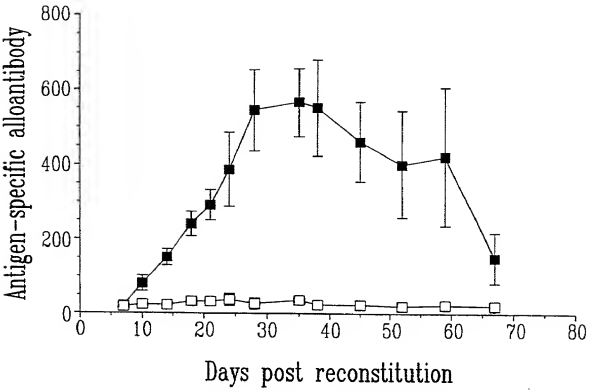

FIG. 5

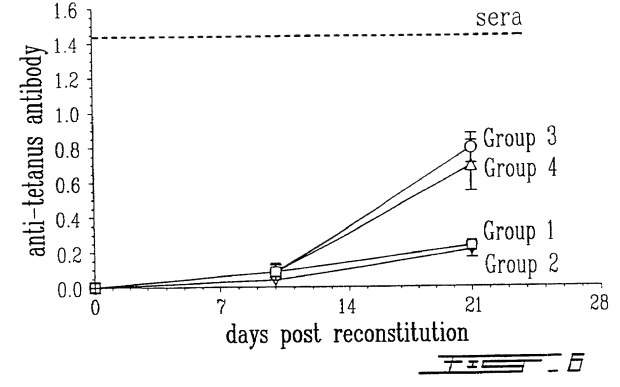

FIG. 6

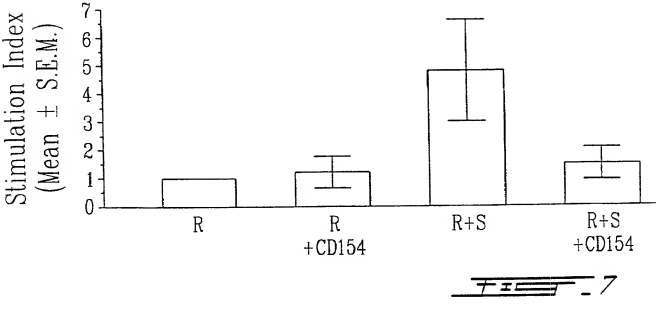

FIG. 7

SEQUENCE LISTING

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LAZARUS, Alan H.
CROW, Andrew R.
FREEDMAN, John

<120> Method for inhibiting in vivo immune response

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Phe Met Lys Thr Ile Gln Arg Cys Asn Thr Gly Glu Arg Ser Leu Ser
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